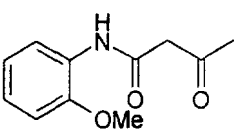
ROBUST SUMMARIES

I. General Information

CAS Number: 92-15-9
Name: Acetoacet-o-anisidide (AAoA)
Acetoacetic acid o-anisidide
Acetoacetyl-o-aniside
o-Acetoacetanisidide (8CI)
2'-Methoxyacetoacetanilide
2-Acetoacetylaminoanisole
2-Methoxyacetoacetanilide
Butanamide, N-(2-methoxyphenyl)-3-oxo- (9CI)
N-(2-Methoxyphenyl)-3-oxobutanamide
o-Methoxyacetoacetanilide

Structure:



II. Physical-Chemical Data

A. Melting Point

Test Substance	
Test substance:	Acetoacet-o-anisidide
Remarks:	Purity unknown
Method	
Method:	Not specified
GLP:	Unknown
Year:	Unknown
Remarks:	
Results	
Melting point value:	86.6 °C
Remarks:	
Data Quality	
Remarks:	
References	Lewis, R.J. (ed.) Sax's Dangerous Properties of Industrial Materials, 8th Edition, Vol. II, Van Nostrand Reinhold, New York, pg. 20, 1992.
Other	

B. Boiling Point

Test Substance	
Test substance:	Acetoacet-o-anisidide
Remarks:	
Method	
Method:	Estimation
GLP:	
Year:	
Remarks:	It was noted in the estimation program that the method was an adapted "Stein and Brown"
Results	
Boiling point value:	375.47 °C
Remarks:	Since the material is a solid, data are technically not needed.
Data Quality	
Remarks:	
References	MPBPWIN v1.3 1; Meylan, W. (1993). User's Guide for the Estimation Programs Interface (EPI), Version 1.2, Syracuse Research Corporation, Syracuse, New York 13210.
Other	

C. Vapor Pressure

Test Substance	
Test substance:	Acetoacet-o-anisidide
Remarks:	
Method	
Method:	Estimation
Remarks:	
Results	
Vapor pressure value:	2.39 x 10" mmHg (2 x 10^{-5} kPa)
Temperature:	25 °C
Remarks:	
Data Quality	
Remarks:	
References	MPBPWIN v I .3 1; Meylan, W. (1993). User's Guide for the Estimation Programs Interface (EPI), Version 1.2, Syracuse Research Corporation, Syracuse, New York 13210.
Other	

D. Partition Coefficient

Test Substance	
Test substance:	Acetoacet-o-anisidide
Remarks:	
Method	
Method:	HPLC procedure
Remarks:	
Results	
Log Pow:	1.01
Remarks:	
Data Quality	
Remarks:	While the detail from the referenced report is relatively scant, it is notable to point out that this **study** was conducted at a very reputable company with an established history of conducting such test. This value is also very close to the **estimated value** of 0.53.
References	Basic Environmental Profile for: Acetoacetyl-o-anisidine; Environmental Sciences Section, Health and Environment Laboratories, at Eastman Kodak Company, Rochester, NY; January 3, 1984 (revised October 8, 1984).
Other	

rest Substance	
Test substance:	Acetoacet-o-anisidide
Remarks:	
Method	
Method:	Estimation
Remarks:	
Results	
Log Pow:	**0.53**
Remarks:	
Data Quality	
Remarks:	
References	KOWIN v 1.63; Meylan, W. (1993). User's Guide for the Estimation Programs Interface (EPI), Version 1.2, Syracuse Research Corporation, Syracuse, New York 13210.
Other	

E. Water Solubility

Test Substance	
Test substance:	Acetoacet-o-anisidide
Remarks:	Purity was 99.6%
Method	
Method:	Unknown
Remarks:	
Results	
Value:	3,340 mg/L
Temperature:	Unknown
Description:	Slight (l-10 g/L)
Remarks:	The test was noted to have been performed in distilled water.
Data Quality	
Remarks:	While the detail from the referenced report is relatively scant, it is notable to point out that this study was conducted at a very reputable company with an established history of conducting such test.
References	Basic Environmental Profile for: Acetoacetyl-o-anisidine; Environmental Sciences Section, Health and Environment Laboratories, at Eastman Kodak Company, Rochester, NY; January 3, 1984 (revised October 8, 1984).
Other	
Test Substance	
Test substance:	Acetoacet-o-anisidide
Remarks:	
Method	
Method:	Estimation
Remarks:	
Results	
Value:	14,300 mg/L
Temperature:	25 °C
Description:	Moderate (10- 100 g/L)
Remarks:	A K_{ow} of 0.53 was used in the estimation
Data Quality	
Remarks:	
References	WSKOW v 1.33; Meylan, W. (1993). User's Guide for the Estimation Programs Interface (EPI), Version 1.2, Syracuse Research Corporation, Syracuse, New York 13210.
Other	

III. Environmental Fate Endpoints

Photodegradation

Test Substance	
Test substance:	Acetoacet-o-anisidide
Remarks:	
Method	
Method:	Estimation
Test type:	Atmospheric oxidation
Remarks:	
Results	
Temperature:	25 °C
Hydroxyl radicals reaction	
OH Rate content:	12.7061 x 10^{-12} cm^3/molecule-sec
Half-life:	0.842 Days (12-hr day; 1.5x 1 0^6 OH^-/cm^3)
Half-life:	10.102 hours
Ozone reaction:	No ozone reaction estimation was noted in the results
Remarks:	
Conclusions	
Data Quality	
Remarks:	
References	AOPWIN v 1.88; Meylan, W. (1993). User's Guide for the Estimation Programs Interface (EPI), Version 1.2, Syracuse Research Corporation, Syracuse, New York 13210.
Other	

B. Stability in Water

Test Substance	
Test substance:	Acetoacet-o-anisidide
Remarks:	Purity was >98%
Method	
Method:	Other
Test type:	Stability of AAoA in simulated gastric fluid
GLP:	No
Year:	1985
Duration:	4 hours
Remarks:	The 0.1 and 1 .O mM test solutions were prepared by diluting stock solutions with 0.1 N HCl. The final pH was approximately 1.0. Test solutions were placed into a 37 °C water bath and shaken. Samples were removed for analysis after 0.25, 0.5, 1 and 4 hours. After removal, samples were neutralized with ammonium hydroxide. The sample pH subsequent to neutralization was between 8-9.
Results	
Nominal Conc.:	0.1043 mM / 1.043 mM
Degradation %:	0.25 hrs: 3.45% (0.1007 mM) / 1.63% (1.026 mM) 0.5 hrs: 2.40% (0.10 18 mM) / 0.77% (1.035 mM) 1 hr: 2.88% (0.1013 mM) / 1.05% (1.032 mM) 4 hr: 1.73% (0.1025 mM) / 0.77% (1.035 mM)
Remarks:	In addition to AAoA, this study evaluated, acetoacet-o-toluidide (AAoT), a similar compound, for its ability to be hydrolyzed under these same conditions. While the results were not presented a similar conclusion was drawn.
Conclusions	Material is not readily hydrolyzed under acidic conditions.
Data Quality	
Remarks:	This study was well-documented.
References	CQSD File No.: EE5H044, HAEL No.: 700-8832, Chemicals Quality Services Division at Eastman Kodak Company, Rochester, NY August 6, 1985
Other	

Test Substance	
Test substance:	Acetoacetanilide (AAA)
Remarks:	Purity was >99%
Method	
Method:	OECD: TG- 111
Test type:	Abiotic hydrolysis
GLP:	Yes
Year:	1990
Remarks:	
Results	
Half-life:	$T_{1/2}$ = 13 1.4 days at pH 1.5 and 37 °C
Degradation %:	< 0.5% after 28 days
Remarks:	The main degradation product formed was aniline
Conclusions	Material does not readily undergo hydrolysis.
Data Quality	
Remarks:	This was an OECD Guideline study conducted under GLP assurances by Hoechst AG, Frankfurt am Main, Germany.
References	Appel, M. and Muhlberger, B. (1990) Abiotischer Abbau Hydrolyse als Funktion des pH-Wertes Analytisches Laboratium, Hoechst AG.
Other	The above information was extracted from the robust summary used to support the submission of this chemical in the OECD/SIDS program.

C. Biodegradation

Test Substance	
Test substance:	Acetoacet-o-anisidide
Remarks:	Purity was >99.5%
Method	
Method:	Other
Test type:	Chemical Oxygen Demand (COD)
GLP:	No
Year:	1982
Remarks:	
Results	
Results:	1.86 grams COD/gram of test substance
Remarks:	
Conclusions	
Data Quality	
Remarks:	While the detail from the referenced report is relatively scant, it is notable to point out that this study was conducted by a very reputable company with an established history of conducting these types of studies.
References	Environmental Analytical Services, Chemicals Quality Services Division, at Eastman Kodak Company, Rochester, NY; HS&HFL No. 82-O 105
Other	

Test Substance	
Test substance:	Acetoacet-o-anisidide
Remarks:	Purity was >99.5%
Method	
Method:	Other; Method is similar to OECD: TG-30 1 C: Modified MITI Test.
Test type:	Biochemical Oxygen Demand (BOD)
GLP:	No
Year:	1982
Remarks:	BOD was determined after 5 and 20 days.
Results	
Results:	BOD5 was 0.03 grams BOD/gram of test substance BOD20 was 0.33 grams BOD/gram of test substance
Remarks:	
Conclusions	Substance is not considered readily biodegradable based on its 20-day degradation value not being 60% of the COD.
Data Quality	
Remarks:	While the detail from the referenced report is relatively scant, it is notable to point out that this study was conducted by a very reputable company with an established history of conducting these types of studies.
References	Environmental Analytical Services, Chemicals Quality Services Division, at Eastman Kodak Company, Rochester, NY; HS&HFL No. 82-O 105
Other	

Test Substance	
Test substance:	Acetoacet-o-anisidide
Remarks:	Purity unknown
Method	
Method:	Other
Test type:	2 1 -Day Biodegradation
GLP:	No
Year:	1982
Contact time:	2 1 -Days
Inoculum:	A microbial inoculum of activated sludge from a laboratory scale synthetic sludge unit maintained at an optimum dry weight of 4,500 mg of mixed liquor suspended solids (MLSS) and unchlorinated effluent obtained from an industrial waste water treatment facility.
Remarks:	The biodegradation screening test was carried out in 20 mL vials designed for use with an automated headspace gas chromatograph. In total, 18 replicate vials were prepared for each test solution. Compound was tested at 20 ppm carbon (C). Prior to the test, test solutions were purged with a stream of carbon dioxide-free air for a minimum of 15 minutes. After filling and sealing, all vials were placed in a darkened incubator-shaker at a temperature of 25 ± 2°C. The samples were gently agitated to provide uniform mixing. On Days 0, 3, 7, 14 and 2 1, triplicate vials were removed and acidified by injection of 0.5 mL 2 N phosphoric acid. (The positive control was sampled on Days 0 and 2 1.) Analysis for carbon dioxide using the above headspace measurement gave a linear response when applied to sealed vials containing aqueous sodium carbonate at concentrations ranging from 0.1 to 10.0 mM. Thus, calibration of carbon dioxide measurements was performed on each sampling day by measuring carbonate standard and water blank solutions sealed within separate vials. The calculated mean values of carbonate concentration (average of three samples) were corrected for each type of solution tested by (a) subtracting the results of the negative control from the compound control and (b) subtracting the results of the inoculum control from both the test and positive control results. The percentage of the theoretical carbon dioxide evolved for triplicate test, compound control, and positive control vials was then calculated. (Boatman et *al.,* "A Method for Measuring the Biodegradation of Organic Chemicals." Environmental Toxicology and Chemistry Vol. 5 (1986): pp. 233 - 243.)
Results	
Degradation % at test end:	18.2% as measured by CO_2 evolution and 22.8% when measured as loss of DOC
Classification:	Material is inherently biodegradable based on the 22.8% reduction in DOC, however, it is not readily degraded based on CO_2 evolution results.
Remarks:	
Conclusions	Results indicate material would not be expected to be persistent in the environment.
Data Quality	
Remarks:	

References	Determination of Biodegradability (Biotic Degradation) using an Automated Screening Method. HAEL No. 82-O 105, Environmental Sciences Section, Health and Environment Laboratories, Eastman Kodak Company, Rochester, **NY.**
Other	

Test Substance	
Test substance:	Acetoacet-o-anisidide
Remarks:	Purity unknown
Method	
Method:	OECD: TG-302B (Zahn/Wellens)
Test type:	Inherent biodegradability
GLP:	Unknown
Year:	1989
Remarks:	
Results	
Degradation %:	30% at 3 days and >97% after 10 days
Remarks:	
Conclusions	Substance appears to have readily degraded.
Data Quality	
Remarks:	A significant amount of detail regarding study methods were not present decreasing the reliability of the information. However, it is notable to point out that the study was completed by a very reputable company with an established history of conducting such studies.
References	Unpublished technical report results from Hoechst Chemical Company.
Other	

D. Transport between Environmental Compartments (Fugacity)

Test Substance'	
Test substance:	Acetoacet-o-anisidide
Remarks:	
Method	
Test type:	Estimation
Model used:	Level III Fugacity Model; EPIWIN: EQC from Syracuse Research Corporation
Remarks:	
Results	
Model data and results: Estimated distribution and media concentration (levels III):	Air 1.05% Water 50.7% Soil 48.2% Sediment 0.103%
Remarks:	Physical chemical parameters utilized were: Temperature (25 "C) water solubility (3.34 mg/L), vapor pressure (2.39 x 10^{-6} mmHg), Log Kow (1.01), melting point (86.6 "C), Henry LC (2.46 x 10^{-13} atm-m^3/mole), and Log Koc (1 .O).
Conclusions	
Data Quality	
Remarks:	
References	Meylan, W. (1993). User's Guide for the Estimation Programs Interface (EPI), Version 1.2, Syracuse Research Corporation, Syracuse, New York 132 10. The Level III model incorporated into EPIWIN is a Syracuse Research Corporation adaptation of the methodology described by Mackay et al. 1996; *Environ. Toxicol. Chem.* 15(9), *16* 18- *1626* and *Environ. Toxicoi. Chem.* 1 5(9), 1627-1637.
Other	

IV. Ecotoxicity

A. Acute Toxicity to Fish

Test Substance	
Test substance:	Acetoacet-o-anisidide
Remarks:	Purity was >99%
Method	
Method:	OECD: TG-203, Acute toxicity to fish
Test type:	Static
GLP:	Yes
Year:	1989
Species/strain:	Zebra Fish (Brachydanio rerio)
Analytical monitoring:	Exposure solution, Temperature, pH, acid content
Exposure period:	96-hr (also monitored at 48-hr.)
Remarks:	
Results	
Observations on precipitation:	None noted
Nominal conc.:	0, 100mg/l, 220 mg/L, 500 mg/L
Endpoint value:	LCO: 220 mg/L (48 Hrs), 220 mg/L (96 Hrs.) LC50: 220-500 mg/L (48 Hrs.), 332 mg/l (96 Hrs.) LC 100: none (48 Hrs.), 500 mg/l (96 Hrs.)
Statistical Methods:	Probit analysis
Remarks:	
Conclusions	The LC_{50} value indicates that the test substance would not be classified according to the European Union's labeling directive and would correspond to a "low concern level" according to the U.S. EPA's assessment criteria.
Data Quality	
Reliability:	(1): Reliable without restrictions
Remarks:	This was a well-documented study conducted following established OECD guidelines and GLP assurances.
References	Acetoacet-o-anisidide TTR, Analysis of the acute toxicity in the Zebrafish (Brachydanio rerio); Pharmaceutical Research Toxicology and Pathology, Hoechst AG, Frankfurt am Main, Germany, Study No. 89.0304; 27 February 1989
Other	

B. Acute Toxicity to Aquatic Invertebrates

Test Substance	
Test substance:	Acetoacet-o-anisidide
Remarks:	Purity was >99.5%
Method	
Method:	Other (essentially similar to OECD: TG-202)
Test type:	Acute immobilization
GLP:	No
Year:	1982
Species/strain:	Daphnia magna
Analytical monitoring:	Aliquots of exposure solution were submitted for concentration determinations at 0 and 96. Temperature, dissolved oxygen, and pH were also determined at these same time periods.
Test details:	96-hour exposure period; static
Remarks:	Water was filter-treated lake water with residual chlorine chemically removed, 10 *Daphnia* were used per dose subsequent to acclimation, exposure temperature ranged from 18-19 °C, pH was 7.7-8.3, and dissolved oxygen was 7.4-9.1 mg/L, Observation for effects were conducted at 24, 48, 72, and 96 hours.
Results	
Nominal conc.:	8.5 and 85 mg/L
Measured conc.:	8.5 and 85 mg/L
Endpoint value:	96-hour EC_{50} > 85 mg/L
Biological observations:	***The Daphniu*** exhibited behavior comparable to controls at all test concentrations at all observation time periods.
Statistical Methods:	NA (No toxicity was observed at highest dose)
Remarks:	
Conclusions	The 96-hour EC_{50} value indicates that the test substance would not be classified according to the European Union's labeling directive and would correspond to a "low concern level" according to the U.S. EPA's assessment criteria.
Data Quality	
Reliability:	(2): Reliable with restrictions
Remarks:	This was a well-documented study conducted by the Environmental Sciences Section, Health and Environment Laboratories, at Eastman Kodak Company, Rochester, NY.
References	HS&HFL No. 82-O 105; June, 1982
Other	

C. Toxicity to Aquatic Plants

Test Substance	
Test substance:	Acetoacet-o-anisidide
Remarks:	
Method	
Method:	Estimation
Test type:	96-hour
Remarks:	
Results	
EC_{50}:	2057.8 1 mg/L
Remarks:	
Conclusions	The results of this estimation indicate that the test substance would not be classified according to the European Union's labeling directive and would correspond to a "low concern level" according to the U.S. EPA's assessment criteria.
Data Quality	
Reliability:	(2) Reliable with restrictions
Remarks:	
References	ECOSAR; Meylan, W. (1993). User's Guide for the Estimation Programs Interface (EPI), Version 1.2, Syracuse Research Corporation, Syracuse, New York 13210.
Other	

Test Substance	
Test substance:	Acetoacetanilide (AAA)
Remarks:	Purity was 99.8%
Method	
Method:	OECD: TG-201
Test type:	Growth inhibition of algae
GLP:	Yes
Year:	1995
Species/strain:	*Selenastrum capricornutum*
Endpoint basis:	Cell growth rate
Exposure period:	72-hours, static test condition
Analytical procedures:	**Yes**
Remarks:	The test was conducted in triplicate in 250 ml Erlenmeyer flasks using a cell density of 10,000 cells/ml. Flasks were shaken continuously at 100 rpm. Temperature was assessed at 0, 24, 48, and 72. The pH was determined at initiation and at study termination, while light flux was assessed on Day 0. Cell density was assessed every 24 hours by removing approx. 2 ml and counting them on a hemacytometer. The minimum quantifiable cell density was 1,000 cells/ml. Observations on gross morphology were assessed.
Results	
Nominal conc.:	11.3, 22.5, 45, 90, 180,360, and 720 mg/L
Endpoint value:	EC_{50} (24-hr) = >720 mg/L EC_{50} (48-hr) = 362 mg/L EC_{50} (72-hr) = 3 18 mg/L NOEC 72-hr = 180 mg/L
Biological observations:	No deformed cells were noted
Was control response satisfactory:	Yes, (a 66 fold increase in density occurred)
Statistical Methods:	Cell density and AUC were evaluated for normality and homogeneity using Chi-square and Bartlett's test with statistical significance determined using Dunnett's.
Remarks:	Temperature ranged from 24.4 – 25.2 °C hours. Average light intensity was 6980 lux, pH was 7.4 at Day 0 and ranged from 7.5 – 9.1 after 72 hours.
Conclusions	The 72-hour EC_{50} values indicate that the test substance would not be classified according to the European Union's labeling directive and would correspond to a "low concern level" according to the U.S. EPA's assessment criteria.
Data Quality	
Reliability:	(1): Reliable without restrictions
Remarks:	This study was an OECD guideline study conducted under GLP assurances by a reputable contract laboratory.
References	LONZA, Inc. Report 1000-0005. Roberts, C.A. and Swigart, J.P. (1995). An Evaluation of Acetoacetanilide in a 72-Hour Toxicity Test with the Freshwater Alga (*Selenastrum capricornutum)*. Wildlife International Ltd.
Other	

V. Toxicological Data

A. Acute Toxicity

Test Substance	
Test substance:	Acetoacet-o-anisidide
Remarks:	Purity was 99.8%
Method	
Method:	EEC Directive 84/449/EEC (OJ No. L25 1, 19.09.84), Part B, Method B1 Acute toxicity (oral)
Test type:	LD_{50} estimate
GLP:	Yes
Year:	1990
Species/strain:	Rat/Crl:CD(SD)
Sex:	Both
Animals/sex/dose:	5
Vehicle:	1% Methylcellulose
Route of exposure:	Oral
Remarks:	Based on a preliminary range fmding study doses of 1260, 1600, and 2000 mg/kg were administered. Animals were approximately 4-6 weeks of age and ranged in weight from 108 -135 grams.
Results	
Value:	
Deaths at each dose:	LD_{50} = 1,637 mg/kg 1260 mg/kg: 1 male (Day 3) 1600 mg/kg: 3 males (Day 2), 2 females (Day 2)
Remarks:	2000 mg/kg: 3 males (Days 1 and 2), 5 females (Days 1,2, and 3) Autopsy of rats that died revealed pale kidneys, pale and patchy livers, congested lungs and congested blood vessels in the small intestine in two males and one female rat dosed at 2000 mg/kg, and a pale spleen and congested blood vessels in the large intestine in two male rats dosed at 2000 mg/kg. No other macroscopic abnormalities were observed. Clinical signs seen in all rats included piloerection, decreased respiratory rates, ptosis, and pallor of extremities. A majority of the rats also exhibited a hunched posture, an abnormal gait, lethargy, and a collapsed state. Recovery was complete by Day 3 in males dosed at 1260 mg/kg and in rats given 1600 mg/kg; Day 4 in 2000 mg/kg exposed animals, and by Day 5 in females exposed at 1260 mg/kg. Except for one low dose female, body weight gain was not affected. Terminal autopsies were unremarkable.
Conclusions	Material would be classified as slightly toxic.
Data Quality	
Reliability:	(1): Reliable without restriction
Remarks:	This is a well-conducted study that followed established guidelines and contained GLP assurances.
References	Lonza report No. 1338. Acute oral toxicity to rats of POO04. HRC Report No. 90339D/LZA 38/AC; Huntingdon Research Center Ltd., Cambridgeshire, England.
Other	

B. Repeated Dose Toxicity

Test Substance	
Test substance:	Acetoacet-o-anisidide
Remarks:	Purity was >99.5%
Method	
Method:	Other
Test type:	14-Day dietary exposure
GLP:	No
Year:	1982
Species/strain:	Rat/Unknown
Route of exposure:	Test material was mixed in diet
Duration of test:	14-Days
Dose levels:	0, 0.1 and 1 .O% (75 and 709 mg/kg)
Sex:	Unknown
Control group and treatment:	1% corn oil was mixed in diet
Post-exposure observation period:	None
Remarks:	Five rats were fed an *ad libitum* diet containing test material. Parameters evaluated included: body weight gain, feed intake, clinical signs, hematology, clinical chemistry, and kidney and liver weight. A specific list of other tissues weighed or examine histologically was indicated (except the spleen).
Results	
NOAEL (NOEL):	A NOEL was not established
Toxic responses by dose:	No mortality or changes in clinical signs were seen at either dose level. While a NOEL was not established, the only effect noted in the low exposure group, attributable to exposure to test compound, was a minor splenic congestion seen in histopathology in 3/5 animals. Treatment-related effects noted in animals exposed to the high dose included slight decreases in body weight gain and serum glucose, and slight increases in BUN and AST. A slight increase in relative liver weight was likely due to the decreases body weight gain, as its absolute value was normal. Hematological changes consisted of decreases in RBC count, hemoglobin, and hematocrit. Changes in RBC appearance consisted of anisocytosis (macrocytes and spherocytes), poikilocytosis, polychromasia, target cells, and Howell-jolly bodies. Enlarged and/or dark spleens were noted in 4 of 5 animals. This gross change was accompanied by a histological observation of minor to moderate congestion in 4 of 5 animals.
Statistical Methods:	Unknown
Remarks:	
Conclusions	The erythron appears to be the main target organ. Essentially all pathological effects noted can be attributed, either directly or indirectly, to toxicity of this tissue. When viewed in context of the studies described below on structurally similar compounds, this study demonstrates that the main target organ for these three chemicals is the same. Accordingly, the 28-day data from the surrogate chemicals should be sufficient for hazard assessment of AAoA following a more chronic exposure.

Data Quality	
Reliability:	(2): Reliable with restriction
Remarks:	While the report for this study lacks a detailed methods section and did not include a comprehensive evaluation of all organ weights or histology examination, it still evaluated a majority of the end-points of a guideline study.
References	Basic Toxicity of Acetoacet-o-Anisidide. Environmental Sciences Section, Health and Environment Laboratories. at Eastman Kodak Company, Rochester, NY. HS&HFL No. 82-0105; TX-82-43
Other	

Test Substance	
Test substance:	Acetoacet-o-toluidide (AAoT)
Remarks:	Purity was 99.93%
Method	
Method:	OECD: TG-422
Test type:	Combined repeat dose and reproductive/developmental toxicity screen.
GLP:	Yes
Year:	1999
Species/strain:	Rat/Crl:CD(SD)
Route of exposure:	Oral gavage
Duration of test:	Males (44 days), Females (14 days before mating to Day 3 of lactation)
Dose levels:	0, 8, 25, 80, 250 mg/kg
Sex:	Both
Control group and treatment:	Controls received vehicle (1% methylcellulose)
Post-exposure observation period:	None
Remarks:	
Results	
NOEL:	25 mg/kg
Toxic responses by dose:	80 mg/kg (males): Hematological examination revealed a decrease in erythrocyte counts, and an increase in MCV. An increase in serum bilirubin was also noted. A blackening of the spleen was seen during gross examination. Histological examination of the spleen and liver revealed the presence of hemosiderin deposits. 250 mg/kg (males): In addition to the effects seen at 80 mg/kg, decreases in hemoglobin concentration and hematocrit values, increases in MCH and reticulocyte counts, a tendency for increase in methemoglobin concentration, and the appearance of Heinz-bodies in erythrocytes were observed. Serum potassium was also noted as being elevated. The absolute and relative weights of the spleen and pituitary were noted as being elevated, however, the pituitary was absent in histopathology. The spleen in this dose group also exhibited extramedullary hematopoiesis and congestion. An increased incidence of eosinophillic bodies was noted in renal proximal tubular epithelial cells. In females, similar pathological changes were detected in the spleen and liver of the two highest dose groups.
Statistical Methods:	Unknown
Remarks:	
Conclusions	The erythron appears to be the main target organ. Essentially all pathological effects noted can be attributed, either directly or indirectly, to toxicity of this tissue.

Data Quality	
Reliability:	(2): Reliable with restrictions
Remarks:	This was an OECD-guideline study conducted under GLP assurances. The study was conducted to meet the requirements for submission of this chemical to the OECD/SIDS program. However, the full report was not available for review.
References	Research Institute for Animal Science in Biochemistry and Toxicity; 3-7-l 1 Hashimotodai, Sagamihara-city, Kanagawa-prefecture, Japan; Study number: 97079
Other	

Test Substance	
Test substance:	Acetoacetanilide (AAA)
Remarks:	Purity was >99%
Method	
Method:	OECD: TG-407
Test type:	28-Day repeat dose toxicity
GLP:	Yes
Year:	1991
Species/strain:	Rat/Sprague-Dawley
Route of exposure:	Single daily oral gavage
Duration of test:	28 Days
Dose levels:	0, 12, 100,850 mg/kg
Sex:	Male and Female
Control group and treatment:	Controls received vehicle (1% methylcellulose)
Post-exposure observation period:	14-days (high-dose only)
Remarks:	
Results	
NOAEL:	12 mg/kg
Toxic responses by dose:	850 mg/kg: Both sexes showed pilo-erection, hunched posture, pallor of the extremities, an abnormal gait, darkened eyes, ptosis, and increased salivation. Except for hunched posture, pilo-erection, and extremity pallor all signs emeliorated during the recovery period. Depressed body weight gains were seen during the treatment period but not during recovery. In males this was accompanied by a reduction in food intake. Increased water consumption was noted during weeks 3 and 6. Hematology results indicated reductions in packed cell volume (PCV), total RBC count, and hemoglobin (Hb) concentration: while increases in mean corpuscular Hb concentration, mean cell volume, and incidence of nucleated red-cells were seen. Red-blood-ceils also exhibited polychromasia, anisocytosis, and a raised methemoglobin level. A leukocytosis was also present. While improvement was noted following recovery, evidence of anemia was still present. Clinical chemistry only revealed an elevated level of bilirubin. Myelograms showed evidence of a regenerative anemia with an increase in erythroid precursors. An increase in liver, spleen, and kidney (male only) weights was noted at termination and after recovery. Spleens were noted as darkened. Microscopic pathology of the liver showed a centrilobular hypertrophy, pigmented Kupffer cells and evidence of extramedullary hematopoiesis. Hemosiderosis was noted in the spleens, and a brownish pigmentation was seen in the kidneys. Male kidneys also contained eosinophillic droplets in the cortical tubules. This pathology persisted through recovery. 100 mg/kg: Animals exhibited pilo-erection, hunched posture, and increased salivation. An abnormal gait was noted in weeks 3 and 4. Hematology, clinical chemistry, and myelogram effects were essentially similar as those noted in the high-dose animals. Spleen and kidney (males only) weights were elevated, and spleens were noted as darkened and microscopically showed hemosiderosis. Brownish pigmentation was noted in the kidneys. 12 mg/kg: In females only, there was a shift in the ratio of erthyroid (increase in normoblast percentage) to myeloid precursor cells indicative of a generation of new RBCs.

Statistical Methods:	Data were analyzed using Bartlett's test for heterogeneity of variance and log-transformed if needed. Homogeneous data was assessed using one-way ANOVA followed by Student's 't' and Williams' test. Nonhomogeneous data was assessed by Kruskal-Wallis analysis of ranks followed by non-parametric equivalent of the 't' test and Williams' test (Shirley's test). The mid- and high-dose finding of increased salivation was only seen in some animals, and was only noted in the post-dosing observation period. The incidence and severity of essentially all pathologies noted occurred in a dose-responsive manner.
Remarks:	
Conclusions	The erythron appears to be the main target organ. Essentially all pathological effects noted can be attributed, either directly or indirectly, to toxicity of this tissue.
Data Quality	
Reliability:	(1): Reliable without restrictions
Remarks:	This was an OECD-guideline study conducted under GLP assurances.
References	LONZA, Inc. Report 1663. Edwards, J.A., Verma, C., Allan, S.A., Crook, D., Gibson, W.A., Suttie, A., Gopinath, C., Anderson, A., Dawe, I.S. (1991). Twenty-Eight Day Oral Toxicity Study in Rats with Acetoacetanilide (POO03). Huntingdon Research Center Ltd.
Other	

C. Genetic Toxicity – Mutation

Test Substance	
Test substance:	Acetoacet-o-anisidide
Remarks:	The test material used in this study was an equal mixture of AAoA supplied by three different manufacturers. It was noted that the chromatogram of the mixture was identical to those of the individual samples. A purity analysis of one manufacturers sample was >99.9%. Because the chromatograms of all three samples were similar it should be assumed that final purity of the mixture was very close to this value too.
Method	
Method:	Other
Test type:	Ames (Salmonella) mutagenicity assay
GLP:	Yes
Year:	1985
Species/strain:	Salmonella typhimurium *TA-98, 100, 1535, I53 7, and 1538*
Metabolic activation:	**Yes**
Concentration tested:	25 .O – 10,000 ug/plate
Remarks:	The study was conducted in triplicate after first determining toxicity potential (+/-S9) using the TA-100 tester strain. The test included both positive and negative (DMSO vehicle) controls.
Results	
Result:	No positive responses were induced in any of the tester strains
Cytotoxic conc.:	> 10,000 ug/plate (no evidence of cytotoxicity was seen)
Precipitation conc.:	No precipitate was noted in the report at the maximum concentration tested.
Genotoxic effects	
With activation:	Negative
Without activation:	Negative
Statistical Methods:	The mean and standard deviation for the number of revertants/plate were determined. Positive and Negative controls were within historical ranges. The report did not discuss how data were analyzed; however, it is obvious that the mean number of revertants was not altered following exposure to test article when compared to controls.
Remarks:	
Conclusions	Material was not genotoxic under conditions of this assay.
Data Quality	
Reliability:	(1): Reliable without restrictions
Remarks:	This was an OECD-like guideline study conducted under GLP assurances by the Environmental Sciences Section, Health and Environment Laboratories, at Eastman Kodak Company, Rochester, NY.
References	Evaluation of Acetoacet-o-Anisidide Blend in the Salmonella Microsome Mutagenicity Assay; HAEL No. 85-0002, TX-85-12; February 1985.
Other	

Test Substance	
Test substance:	Acetoacet-o-anisidide
Remarks:	The test material used in this study was an equal mixture of AAoA supplied by three different manufacturers. It was noted that the chromatogram of the mixture was identical to those of the individual samples. A purity analysis of one manufacturers sample was >99.9%. Because the chromatograms of all three samples were similar it should be assumed that final purity of the mixture was very close to this value too.
Method	
Method:	Other; O'Neill, J.P., *et al. Mutation Research* 45, 91- 10 1, 1977.
Test type:	CHO/HGPRT Forward Mutation Assay
GLP:	Yes
Year:	1985
Species/strain:	Chinese hamster ovary cells/CHO-K 1 -BH4
Metabolic activation:	**Yes**
Concentration tested:	1 .0-3 .0 mg/ml
Remarks:	Briefly, Cells are maintained in a hypoxanthine-free culture media. The study utilized a negative control of DMSO and a positive control of ethyl methanesulonate (-S9) and dimethylnitrosamine (+S9). After an overnight incubation, test material (and S9) is added to flask containing about 5 x 10^5 cells. Exposures last for 4-hours, after which the cultures are washed and incubated over night. Cells are then trypsinized and reseeded at approximately 100 cells/flask. They then undergo a 7-day incubation. Colonies are then harvested, stained to determine viability, and reseeded at 10^6 cells/flask. Over the next 2-3 days, colonies are subcultured (10^6/flask) and incubated another 8-12 days. After this expression period each culture is reseeded into petri dishes (5-12 dishes) at 2 x 10^5 cells and incubated for 7 more days with mutant selection media. Other cultures are also set up to assess cloning efficiency.
Results	
Result:	A significant increase in mutant colonies was not observed (see remarks).
Cytotoxic conc.:	3 mg/ml
Precipitation conc.:	No precipitate was noted in the report.
Genotoxic effects	
With activation:	Negative
Without activation:	Negative
Statistical Methods:	Conclusions concerning whether the mutation frequencies at each dose level were significant was based on Kastenbaum and Bowman (Tables of determining the statistical significance of mutation frequencies, *Mutation Research 9,* 527-549, 1970)
Remarks:	One positive mutant response was noted in a –S9 sample at 2.5 mg/ml. However, the response was minimal (24.3 mutants/10^6 cells verse 242.4 for the positive control and an average of 8.45 for the vehicle and media controls) and could not be repeated in a second trial. Furthermore, a true positive response should exhibit a dose-response and such an effect was not observed. All parameters in the primary and repeat study met assay acceptance criteria.
Conclusions	Material was not genotoxic under conditions of this assay.

Data Quality	
Reliability:	(1): Reliable without restrictions
Remarks:	This was a well-documented study conducted under GLP assurances by the Environmental Sciences Section, Health and Environment Laboratories, at Eastman Kodak Company, Rochester, NY.
References	Evaluation of Acetoacet-o-Anisidide in the CHO/HGPRT Forward Mutation Assay; HAEL No. 85-0002, TX-85-19; February 1985.
Other	

D. Genetic Toxicity – Chromosomal Aberrations

Test Substance	
Test substance:	Acetoacetanilide (AAA)
Remarks:	Purity was 99.7%
Method	
Method:	OECD: TG-473
Test type:	Cytogenetics assay in peripheral human blood lymphocytes
GLP:	Yes
Year:	1990
Route of exposure:	*In vitro*
Concentration tested:	232,929, and 1860 ug/ml (-S9) and 464, 1860, and 3710 ug/ml (+S9)
Metabolic activation:	Yes; Aroclor 1254 induced rat liver S9
Remarks:	RPMI 1640 culture media, DMSO vehicle, fixation time was 48 hours, 1 plate/test and 2 replicates, positive control was ethylmethane sulphonate (-S9) and cyclophosphamide (+S9)
Results	
Result:	No significant increases in cells with chromosomal aberrations were observed.
Cytotoxic conc.:	3710 ug/ml (-S9); >3710 ug/ml (+S9)
Precipitation conc.:	5000 ug/ml (3710 ug/ml was max. concentration not causing precipitation)
Genotoxic effects	
With activation:	Negative
Without activation:	Negative
Statistical Methods:	Fisher's Test
Remarks:	Both positive controls induced large statistically significant increases in the proportion of aberrant cells
Conclusions	Material was not genotoxic under conditions of this assay.
Data Quality	
Reliability:	(1): Reliable with restrictions
Remarks:	This was a well-documented OECD guideline study conducted under GLP assurances at Huntingdon Research Center Ltd., England
References	Lonza, Inc. Report 1530. Brooker, P.C., Paterson, K.M.A., and King, J.D. Metaphase chromosome analysis of human lymphocytes cultured in vitro.
Other	

Test Substance	
Test substance:	Acetoacet-o-toluidide (AAoT)
Remarks:	Purity was 99.93%
Method	
Method:	Guidelines for screening mutagenicity testing of chemicals (Japan) and OECD: TG-473
Test type:	Chromosomal aberrations in cultured lung cells
GLP:	Yes
Year:	1999
Species/strain:	Chinese hamster
Route of exposure:	In vitro
Concentration tested:	Up to 5000 ug/ml
Metabolic activation:	Yes; Phenobarbital and 5,6-benzoflavone induced rat liver S9
Remarks:	DMSO vehicle, positive controls consisted of cyclophosphamide and mitomycin C, 2 plates/test.
Results	
Result:	Structural chromosomal aberrations (including gaps) were induced under the following conditions: 24 hr continuous treatment (2.5 mg/ml, 10.0%); 48 hr continuous treatment (1.8 mg/ml, 5.0%); short-term treatment without S9 mix (5 mg/ml, 9.0%); short-term treatment with S9 mix (5 mg/ml, 5.0%). The confirmative examination was conducted with 24-hr continuous treatment, because structural aberrations were only induced at the dosage of 2.5 mg/ml. As a result, structural chromosomal aberrations were induced dose-dependently. Polyploidy was not induced under any test conditions.
Cytotoxic conc.:	3.5 mg/ml with 24 hr continuous treatment and 3.6 mg/ml with 48 hr continuous treatment.
Precipitation conc.:	Precipitation was not noted in the summary
Genotoxic effects	
With activation:	Equivocal
Without activation:	Positive (2.5 mg/ml continuous treatment; clastogenicity)
Statistical Methods:	Unknown
Remarks:	
Conclusions	Material was genotoxic in the absence of metabolic activation under conditions of this assay but was not positive when a metabolic activation system was present.
Data Quality	
Reliability:	(2): Reliable with restrictions
Remarks:	This was an OECD-guideline study conducted under GLP assurances. The study was conducted to meet the requirements for submission of this chemical to the OECD/SIDS program. However, the full report was not available for review.
References	Biosafety Research Center, Foods, Drugs and Pesticides; 582-2 Arahama, Shioshinden, Fukude-cho, Iwata-gun, Shizuoka, Japan; Study number: 3649 (115-082)
Other	

E. **Genetic Toxicity – Primary DNA Damage**

Test Substance	
Test substance:	Acetoacet-o-anisidide
Remarks:	The test material used in this study was an equal mixture of AAoA supplied by three different manufacturers. It was noted that the chromatogram of the mixture was identical to those of the individual samples. A purity analysis of one manufacturers sample was >99.9%. Because the chromatograms of all three samples were similar it should be assumed that final purity of the mixture was very close to this value too.
Method	
Method:	Other; Williams, G. (1977) Detection of chemical carcinogens by unscheduled DNA synthesis in rat liver primary cell cultures. *Cancer Research 37,* 1845-1851.
Test type:	Unscheduled DNA synthesis test
GLP:	Yes
Year:	1985
Species/strain:	Rat/CD- 1
Route of exposure:	*In vitro*
Concentration tested:	Up to 3.2 mg/ml
Metabolic activation:	NA (hepatocytes were utilized in the test assay)
Remarks:	Positive control was 2-aminoanthracene and DMSO was used for a negative vehicle control. Livers were excised from rats while under Metofane® anesthesia and hepatocytes were harvest by mechanical dispersion. Cells are washed and 3.0 ml, of a 1.7 x 10^5 suspension, is placed in culture dishes (5 dishes/dose). Two of the dishes are used for cytotoxicity determination. Cultures containing test material and tritiated-thymidine are incubated for 18 hours. After exposure, nuclei are swollen with Na-citrate, cultures are fixed with acetic acid/ethanol and air-dried over night on cover slips. Autoradiographs are prepared by dipping the mounted cover slips into nuclear track emulsion where, following a 10-day "incubation period, are developed and stained. Fifty randomly selected cells per slide are counted for net nuclear grain count.
Results	
Result:	No significant increase in UDS was observed.
Cytotoxic conc.:	3.20 mg/ml (58.7% survival)
Precipitation conc.:	None noted
Cenotoxic effects	
With activation:	Negative
Without activation:	NA
Statistical Methods:	Tests were considered positive if there was 1.) An increase in the mean net nuclear grain (NNG) count of at least five grains per nucleus in excess of the concurrent negative control; or 2.) The percentage of nuclei with five or more NNG is at least 10% higher than the concurrent negative control; or 3.) The percentage of nuclei with 20 or more NNG is at least 2% higher than the concurrent negative control population.
Remarks:	
Conclusions	Material was not genotoxic under conditions of this assay.

Data Quality Reliability: Remarks:	(1): Reliable without restrictions This was a well-documented study conducted under GLP assurances,
References	Evaluation of Acetoacet-o-Anisidide Blend in the Unscheduled DNA synthesis test; HAEL No. 85-0002, TX-85-14; Environmental Sciences Section, Health and Environment Laboratories, at Eastman Kodak Company, Rochester, NY. March 1985.
Other	

F. Developmental Toxicity

rest Substance	
Test substance:	Acetoacetanilide (AAA)
Remarks:	Purity was >99%
Method	
Method:	OECD: TG-42 1
GLP:	Yes
Year:	1996
Species/strain:	Rat/Sprague-Dawley
Sex:	Both
Route of exposure:	Oral gavage
Dose levels:	0, 3, 30, 100 mg/kg
Exposure period:	Males were treated for 6 weeks beginning 14 days prior to breeding; Females were treated from 2 weeks before breeding through Day 4 of lactation.
Frequency of treatment:	Once per day
Control group and treatment:	1% methylcellulose
Remarks:	10 animals/sex/dose
Results	
Maternal toxicity NOEL:	3 mg/kg
Repro./Develop. Toxicity NOEL:	100 mg/kg
Paternal/Maternal toxic responses by dose:	3 mg/kg: No adverse effects were noted 30 mg/kg: Methemoglobinemia was noted in both males and females. 100 mg/kg: Excess salivation was noted in 5/10 males. Body weight and feed consumption were reduced during days 1-8 of the pre-breeding period. Females showed decreased weight gain during gestation. Methemoglobinemia was noted in both males and females, as was an increase in white cell count. Reductions in red cell count, hemoglobin, and hematocrit were noted, while increases in mean cell volume and mean corpuscular hemoglobin content were recorded. Spleen weights were increased.
Fetal toxic responses by dose:	No effects were noted on any mating or fertility parameter at any dose level. No effects were noted on any fetal parameter evaluated.
Statistical Methods:	Data were analyzed using Bartlett's test for heterogeneity of variance followed by one-way ANOVA and Dunnett's test. Nonhomogeneous data was assessed by Kruskal-Wallis and Dunn's test or Fisher's exact test.
Remarks:	
Conclusions	Test material did not induce reproductive or developmental toxicity under the conditions of this assay.
Data Quality	
Reliability:	(1): Reliable without restrictions
Remarks:	This was a well-documented OECD guideline study conducted under GLP assurances.

References	Oral (Gavage) Reproductive/Developmental Toxicity Screen of Acetoacetanilide in Rats (OECD Guideline 42 1); Laboratory Project ID 720-003; Argus Research Laboratories Inc., Horsham, PA.
Other	

Test Substance	
Test substance:	Acetoacet-o-toluidide (AAoT)
Remarks:	Purity was 99.93%
Method	
Method:	OECD: TG-422; Combined repeat dose and reproductive/developmental toxicity screen.
GLP:	Yes
Year:	1999
Species/strain:	Rat/Crl:CD(SD)
Sex:	Both
Route of exposure:	Oral gavage
Dose levels:	0, 8, 25, 80,250 mg/kg
Exposure period:	Males (44 days), Females (14 days before mating to Day 3 of lactation)
Frequency of treatment:	Once per day
Control group and treatment:	Controls received vehicle (1% methylcellulose)
Remarks:	
Results	
Maternal toxicity NOEL:	25 mg/kg (both males and females)
Repro./Develop. Toxicity NOEL:	>250 mg/kg
Paternal/Maternal toxic responses by dose:	80 mg/kg (males): Hematological examination revealed a decrease in erythrocyte counts, and an increase in MCV. An increase in serum bilirubin was also noted. A blackening of the spleen was seen during gross examination. Histological examination of the spleen and liver revealed the presence of hemosiderin deposits. 250 mg/kg (males): In addition to the effects seen at 80 mg/kg, decreases in hemoglobin concentration and hematocrit values, increases in MCH and reticulocyte counts, a tendency for increase in methemoglobin concentration, and the appearance of Heinz-bodies in erythrocytes were observed. Serum potassium was also noted as being elevated. The absolute and relative weights of the spleen and pituitary were noted as being elevated, however, the pituitary was absent in histopathology. The spleen in this dose group also exhibited extramedullary hematopoiesis and congestion. An increased incidence of eosinophillic bodies was noted in renal proximal tubular epithelial cells. In females, similar pathological changes were detected in the spleen and liver of the two highest dose groups. There were no effects noted on any mating or fertility parameter at any dose level.
Fetal toxic responses by dose:	No effects were noted on any fetal parameter evaluated.
Statistical Methods:	Unknown
Remarks:	
Conclusions	Test material did not induce reproductive or developmental toxicity under the conditions of this assay

Data Quality	
Reliability:	(2): Reliable with restrictions
Remarks:	This was an OECD-guideline study conducted under GLP assurances. The study was conducted to meet the requirements for submission of this chemical to the OECD/SIDS program. However, the full report was not available for review.
References	Research Institute for Animal Science in Biochemistry and Toxicity; 3-7- 11 Hashimotodai, Sagamihara-city, Kanagawa-prefecture, Japan; Study number: 97079
Other	

G. Toxicity to Reproduction

This endpoint was satisfied through the studies on AAA and AAoT summarized in the above section **on** developmental toxicity, as these studies also a screened for reproductive toxicity.